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UV- VISIBLE SPECTROPHOTOMETER METHOD IN DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LEFLUNOMIDE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, accurate, precise, reproducible, highly sensitive, an economic spectrophotometric method has been developed for the estimation of Leflunomide. UV-Visible spectrophotometric method is based on the measurement of absorption at a maximum wavelength of 320 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision (inter and intraday variations). Beer's law was obeyed in the concentration range of 5–25 µg/mL with a correlation coefficient of 0.9994. Results of the analysis were validated statistically and by recovery study. Hence the developed and validated method can be used for estimation of Leflunomide.

INTRODUCTION

Leflunomide is a disease- modifying antirheumatic drug (DMARDs) which is FDA approved to treat individuals with rheumatoid arthritis. It is a non-biological novel isoxazole derivative that has demonstrated that has demonstrated anti-inflammatory characteristics.

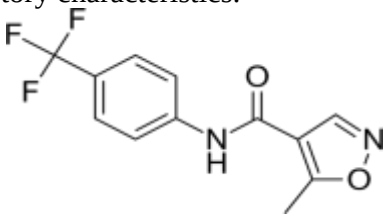

Fig.-01- Leflunomide

Leflunomide is used to relieve symptoms caused by active rheumatoid arthritis, such asinflammation, swelling, stiffness, and joint pain. This medicine works by stopping the body from producing too many of the immune cells that are responsible for the swelling and inflammation. Leflunomide (Arava) is a drug approved to treat adult moderate to severe rheumatoid arthritis along with other rheumatic diseases. It belongs to a class of medications called disease-modifying antirheumatic drugs (DMARDs), which aim to decrease inflammationand permanent damage.

MATERIAL AND METHOD:

Instrumentation: Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis finasteride and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Sonicator (1.5L) Ultrasonicator was used to sonicating the standard and formulation sample. Standard and

sample drugs were weighed by using Denver electronic analytical balance (SI-234).

Reagents standard and samples: Working standard sample Leflunomide was obtained from well reputed research laboratory, formulation sample was purchased from local pharmacy. Spectrophotometric reagents i.e. methanol and colouring reagents like erichromo black t, methyl blue, bromocresol green and bromo thymol blue was purchased.

Procedure

Preparation of standard stock solution: Standard stock solution of Leflunomide pure drug was prepared by accurately weighing about 10mg of each drug in 10ml volumetric flask. The drugs were dissolved with 5ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent; results 1000µg/ml solution was obtained. From this 1ml was taken and diluted to 10ml to get a concentration of 100µg/ml. from 100µg/ml solution 2ml was taken and make up to 20ml to get a final working stock solution of 10µg/ml. required concentrations or dilutions need for uv andvisible estimation was prepared from 10µg/ml solution.

Preparation of Formulation Sample: Leflunomide (Topicid-120mg/10ml) sample solution is taken equivalent to 10mg was dissolved in 10ml of Methanol. The solution was sonicated for 10min to complete extraction of drugs in Methanol. The solution was filtered through 0.45µm nylon membrane filter paper.

UV Spectrophotometric estimation:

Selection of solvent for solubility: The drug Leflunomide was practically soluble in Water and absorbance of solution was measured. Finally, dilutions with water were show improved absorbance compared to other solvents. Hence standard drug was soluble in water and necessary required dilutions were prepared with water as diluents for spectrophotometric estimation.

Selection of wavelength maxima: Suitable maximum absorbance for the estimation of Leflunomide was identified by scanning the absorbance in spectrum mode within the wavelength region of 400-200nm in three differentdilute solutions. In all the solutions the drug absorbs maximum wavelength at 220nm. Hence 220nm was found to be suitable wavelength for the estimation of Leflunomide**.**

Construction of calibration curve: From the prepared standard stock solution, a series of calibration standards were prepared by selected dilutions. From the stock solution, 100µg/ml, 200, 300, 400, 500µg/ml was prepared. The absorbance of the prepared solutions was measured at 220nm against a regent blank. At each concentration a triple readings were measured and mean value was used for the Construction of calibration curve. Calibration curve was constructed by taking concentration of the prepared solution on x-axis and corresponding absorbance on y-axis.

Formulation analysis: The absorbance of the prepared formulation solution in all the brands was measured at 220nm in triplets separately. The average absorbance value was used for the formulation estimation ofLeflunomide. The % assay estimated in the prepared sample solutions by substituting the absorbance values in the equation given below.

Visible Spectrophotometric estimation:

Preparation of Reagents: PNA solution: weigh 200 mg of PNA and is dissolved in 100ml of distill water.HCL Solution: dissolve 8.6 ml of concentrated hydrochloric acid in 1000ml of distill water.

Method: In a series of 125 ml separating funnels containing aliquots of standard drug solution was taken.To this 6ml of HCl solution and 2ml of PNA solutions were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 610.5nm against a similar reagent blank.

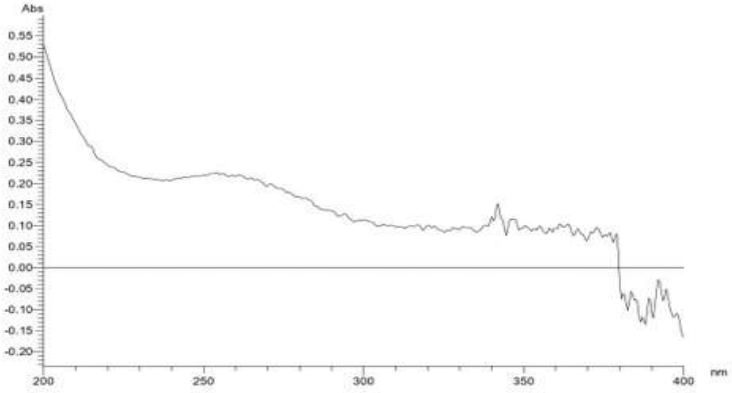

Fig .02 – Wave length scanning spectrum of Leflunomide in UV region

S.NO	Concentration	Average Absorbance
1	10µg/ml	0.083
2	20µg/ml	0.119
3	30µg/ml	0.144
4	40µg/ml	0.175
5	50µg/ml	0.197
6	60µg/ml	0.232

Table. 01- Calibration curve result

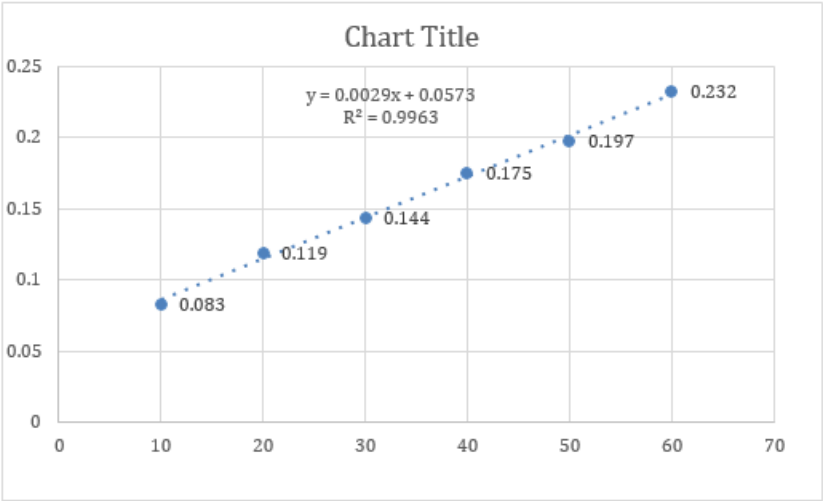

FIG.03-Calibration curve of Leflunomide in UV

S.NO	Brand	Dosage	Amount Prepared	Absorbance Found	%Assay
1	Leflu - 20	20mg	10µg/ml	0.166	94.7%
2	Afonide -10	10mg	20µg/ml	0.169	98%
3	Lefumit-10	10mg	30µg/ml	0.171	96.2%

Table.02- Formulation result in UV

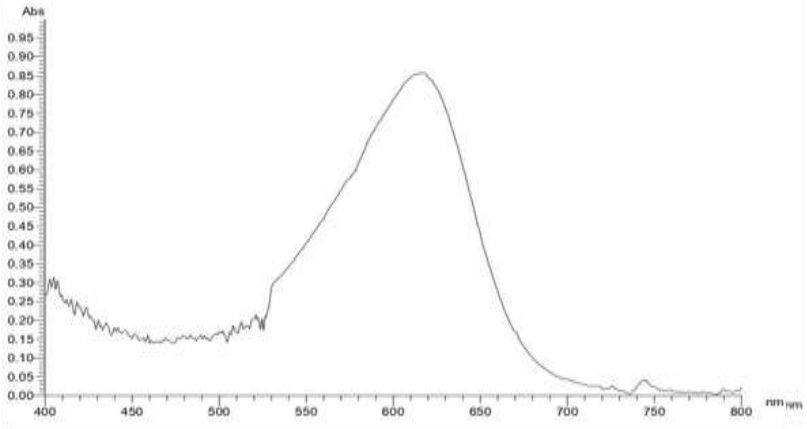

Fig.04- wavelength scanning spectrum of leflunomide in visible region

S.No	Concentration	Average Absorbance
1	0.2µg/ml	0.085
2	0.48µg/ml	0.108
3	0.68µg/ml	0.136
4	0.8µg/ml	0.168
5	1µg/ml	0.197
6	1.2µg/ml	0.225
7	Slope Intercept Correlation Coefficient	0.9982

Table.03-Calibration curve results

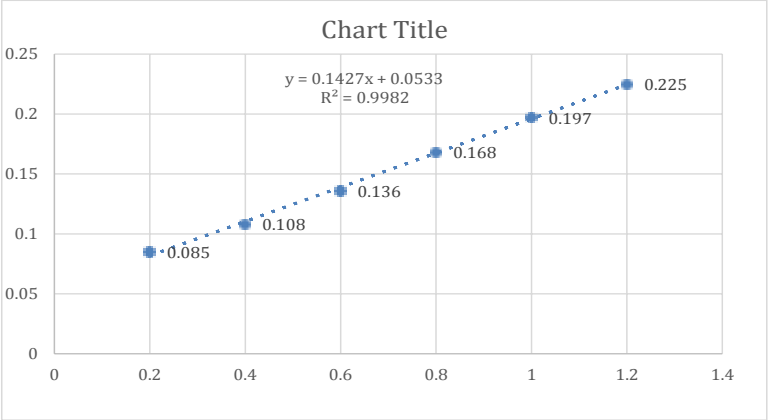

Fig. 05- Calibration curve of Finasteride in Visible method

Organic layer attains orange redcolor while the blank is colorless.

Formulation Assay: From the prepared 10µg/ml of the sample solution, 1ml was taken and the method procedure as describes above was applied. After the development of the color, the absorbance of the separated chloroform layer was measured at 215nm against a similar reagent blank. The resultant absorbance values were used for the estimation of Leflunomide in the formulation assay.

RESULT AND DISCUSSION:

UV- Method: Wavelength maximum were identified for the leflunomide drug at dilute concentration. Specific wavelength maximum was identified at a wavelength of 320.5. Hence 320.5 were found to be most suitable wavelength for the estimation of finasteride

Wavelength scanning result was shown in figure-02. Good linear relation was observed within the prepared concentrations of 10-60µg/ml regression equation was found to be y = 0.0029x + 0.0573 with correlation coefficient of 0.9963. Results of calibration curve was shown in fig.03 and table.01

Formulation Assay: The absorbance of the prepared formulation solutions was measured and from the resultant sample values % assay was calculated. In the estimation of Leflunomide. Results of the essay studies were shown in table 02

Visible Spectrophotometer estimation: From the prepared colour developed solution of the leflunomide one solution was taken and the absorbance of the solution was scanned in the visible region i.e. 800nm-400nm against a similarly prepared reagent blank. At a wavelength of 727.0nm was found to be most suitable for the estimation of Leflunomide. Wavelength scanning spectrum was shown in figure-4. Six points calibration curve was constructed with in the concentration range of 0.2-1.2µg/ml. regression equation was found to be y = 0.004x -0.009 with a correlation of 0.996. Results of the calibration curve were shown in table 3 and calibration curve was shown in figure-5

Formulation Assay: The absorbance of the prepared formulation solutions was measured and from the resultant sample the absorbance of the prepared formulation solutions was measured and from the resultant sample values % assay was calculated. Results of the essay studies were shown in table 4

CONCLUSION:

Leflunomide is used to relieve symptoms caused by active rheumatoid arthritis, such as inflammation, swelling, stiffness, and joint pain. This medicine works by stopping the body from producing too many of the immune cells that are responsible for the swelling and inflammation. Leflunomide (Arava) is a drug approved to treat adult moderate to severe rheumatoid arthritis along with other rheumatic diseases. It belongs to a class of medications called disease-modifying antirheumatic drugs (DMARDs), which aim to decrease inflammation and permanent damage. In UV region drug was estimated at 220nm using methanol as diluents and in visible region the color was developed using Para-nitro aniline reagent. The maximum absorbance of the developed red color was found to be 610nm. Beers law equation was found to be y = 0.0001x -0.082 for UV and y = 0.004x - 0.009 for Visible method. In both these methods the drugs was estimated more than 98% assay.

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